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mercaptoethanol, pH 8.0) and is kept at 37°C after addition of 180 units of ExoIII nuclease. Ten μl portions of the solution are sampled every 30 seconds and transferred to tubes containing 10 μl of MB buffer(40 mM NaCl, 2 mM $ZnCl_2$, 10% glycerol, pH 4.5) in an ice bath. After sampling, the 10 tubes are kept at 65°C for 10 minutes to inactivate the enzyme. Then, 5 units of mung bean nuclease is added and kept at 37°C for 30 minutes. From one original plasmid, ten different kind of DNA fragments are recovered by agarose gel electrophoresis. The degree of deletion of each fragment varies. The terminals of the recovered DNAs are smoothed with Klenow enzyme to subject to ligation reaction at 16°C overnight, and by using resulting DNA, E. coli DH5α is transformed to obtain clones. The plasmids are prepared from the various clones obtained, and nucleotide sequences are determined by using luminescence primer cycle sequence kit(Applied Biosystems corp.) with an automatic sequencer.

As a result, it was found that the nucleotide sequence of the cDNA in pRH1 derived from Phaffia rhodozyma consists of 1,099 base pairs (~~SEQUENCE ID No. 4~~ SEQ ID NO:4), and there is an open reading frame which encodes a polypeptide having 251 amino acids (SEQ ID NO:1, and 2 which corresponds the region from A to B in Figures 4 and 5). It was also found that the nucleotide sequence of the cDNA in pHP1 derived from Haematococcus pluvialis consists of 1,074 base pairs (~~SEQUENCE ID No. 5~~ SEQ ID NO:5), and there is an open reading frame which encodes a polypeptide having 259 amino acids (SEQ ID NO:2 which corresponds the region from C to D in Figures 6 and 7). The amino acid sequences expected from these open reading frames are investigated by analyzing homology in the Gene Bank. Both of the amino acid sequences of Ph. rhodozyma and Ha. pluvialis have

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(EXAMPLE 9) Isolation of the IPP isomerase gene of Saccharomyces cerevisiae by PCR method

Based on the nucleotide sequence of the IPP isomerase gene of S. cerevisiae reported in the aforementioned reference(Anderson, M. S., Muehlbacher, M., Street, I.P., Profitt, J., Poulter, C. D., "Isopentenyl diphosphate: dimethylallyl diphosphate isomerase - an improved purification of the enzyme and isolation of the gene from Saccharomyces cerevisiae", J. Biol. Chem., 264:19169-19175(1989)), the primers (SEQ ID NOS 7 and 8, respectively) below were synthesized.

Primer No. 1 5'-TCGATGGGGGTTGCCTTTCTTTTTCGG-3'

Primer No. 2 5'-CGCGTTGTTATAGCATTCTATGAATTTGCC-3'

The procedure was designed to obtain PCR amplified IPP isomerase gene having TaqI sites at the upstream terminal and AccII region at the downstream terminal. Thirty cycles of PCR is performed with 200 ng of total DNA of S. cerevisiae and PfuDNA polymerase (STRATAGENE). To express the IPP isomerase gene obtained by PCR in E. coli, it is digested with both TaqI and AccII. Then, the gene was inserted into ClaI sites and SmaI sites of pBluescript KS+ vector. The resulted plasmid was named pSI1(Figure 11). This DNA derived from S. cerevisiae had a nucleotide sequence consisting of 1,058 bp (~~SEQUENCE ID No. 6~~ SEQ ID NO: 6), and contained a gene which encodes IPP isomerase consisting of 288 amino acids(corresponds from E to F in Figures 8 and 9). (SEQ ID NO: 3)

(EXAMPLE 10) Increase of lycopene production amount by introducing the IPP isomerase gene

Into the lycopene-producing E. coli JM101 strain (abbreviated as L hereafter) which contains pACCRT-EIB(Figure 10), pSPORT1